m-Benziporphodimethene: a new porphyrin analogue fluorescence zinc(II) sensor†

Chen-Hsiung Hung,*[a] Gao-Fong Chang,[a] Anil Kumar,[a] Geng-Fong Lin,[b] Li-Yang Luo,[ac] Wei-Min Ching[a] and Eric Wei-Guang Diau[c]

Received (in Cambridge, UK) 18th September 2007, Accepted 3rd December 2007
First published as an Advance Article on the web 21st December 2007
DOI: 10.1039/b714412a

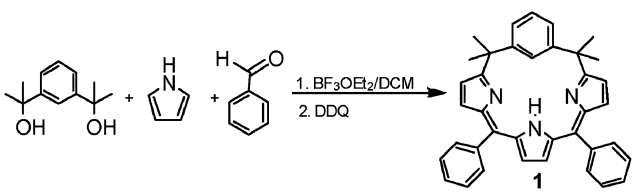
***m*-Benziporphodimethene is presented here as a long-wavelength Zn^{2+} specific chemosensor; this sensor shows fluorescence switch-on upon Zn^{2+} binding with no apparent background fluorescence.**

Fluorescent metal ion sensors have become increasingly important tools for the quantitative, real time monitoring of metal ions in biological samples.[1–10] Metal coordination that increases the fluorescence intensity of the chromophore is called chelation-enhanced fluorescence (CHEF). Most CHEF sensors are based on fluorophores, such as fluorescein,[11,12] dansyl,[13–16] and anthracene,[2,4] which emit at wavelengths shorter than 600 nm. Sensors that are effective above 600 nm, where background fluorescence is minimal, minimize light induced tissue damage, penetrate better and scatter less in optically diffuse samples should be further developed.[17–19]

Zinc is, after iron, the second most abundant transition metal in mammals,[20] where it plays important roles in various biological processes such as neurotransmission, signal transduction and gene expression.[21,22] A large body of knowledge exists on the structural chemistry of zinc, but little is known about zinc homeostasis.[23] This lack of knowledge can be attributed to the spectroscopically silent nature of d^{10} Zn^{2+}. The well-established 8-aminoquinoline-based zinc chemosensors show about a 300-fold increase in fluorescence intensity after zinc chelation.[23] Lippard and co-workers recently reported low background Zn^{2+} induced fluorescence turn-on molecules that were suitable for biological imaging.[24] Protein[25] and peptide based[26] sensing approaches have also emerged. Other sensors including ACF,[27] Rhod–Zn,[28] and ZinPyr,[29] have also been developed, but a majority of the available zinc sensors suffer from high background fluorescence and weak fluorescence enhancement. Herein, we provide a novel *m*-benziporphodimethene based molecule, which imparts no background emission, and, upon Zn^{2+} binding, increases fluorescence intensity. This compound represents a turn-on probe for Zn^{2+} sensing.

11,16-Bis(phenyl)-6,6,21,21-tetramethyl-*m*-benzi-6,21-porphodimethene (**1**), a porphyrin analogue, was synthesized through an acid catalyzed condensation reaction of α,α-dihydroxy-1,3-diisopropylbenzene, pyrrole and benzaldehyde at room temperature, as shown in Scheme 1.[30] Compound **1**, isolated in 27% yield as a red solid, was purified by silica gel column chromatography.‡ Because of the discrete conjugated system, the absorptions in UV-Vis spectrum for **1** is broad. As shown in Fig. 1 (top, black line), a higher energy Soret-like absorption band is observed at 349 nm, while broad low energy bands are apparent in the visible region at 514 and 542 nm. An extinction coefficient of 3.59×10^4 M^{-1} cm^{-1} for π to π* transition at 349 nm is about one order smaller than that of the Soret band for tetraphenylporphyrin.[31] Compound **1** is non-fluorescent in free base form. As the formation of a **Zn·1** complex occurred, after the addition of Zn^{2+}, there was an instant change in solution color from pink to greenish blue, which accompanied the shifting of the visible band from 542 to 639 nm. Complex **Zn·1** formation was confirmed by observing the loss of the inner amino protons by NMR, and by the observation of a mass spectrum peak of m/z 594.1880, corresponding to $[M - Cl]^+$ upon the addition of Zn^{2+} into a solution of **1**.‡ The X-ray single-crystal structure§ of the complex unambiguously confirmed the coordination of zinc ion with benziporphodimethene through three pyrrolic nitrogens and an axial chloride (see ESI†). The structure of the zinc complex gave a co-planar tripyrrin unit in contrast to the distorted benziporphodimenthene core in the free-base form, and this might contribute to its unusual fluorescence turn-on.[30]

Compound **1** has a superb chemosensory response when Zn^{2+} is added. The non-fluorescent solution of the free-base form changes to a red emitting solution with λ_{em} at 672 nm upon the addition of Zn^{2+} (Fig. 1 bottom). The Stokes shift of 33 nm is not large, but is comparable to other chromophores. The fluorescence quantum yield (Φ_F) of 0.34 at the S_1 state in degassed toluene at room temperature for **Zn·1** is calculated



Scheme 1

[a] *Institute of Chemistry, Academia Sinica, Nankang, Taipei 105, Taiwan. E-mail: chhung@chem.sinica.edu.tw; Fax: +886-2-27831237*
[b] *Department of Chemistry, Tamkang University, Tamsui, Taipei 251, Taiwan*
[c] *The Department of Applied Chemistry, National Chiao Tung University, Hsinchu, 300, Taiwan*

† Electronic supplementary information (ESI) available: Synthetic procedures for **1** and **Zn·1**, Job plot, and ORTEP plot of **Zn·1**. See DOI: 10.1039/b714412a

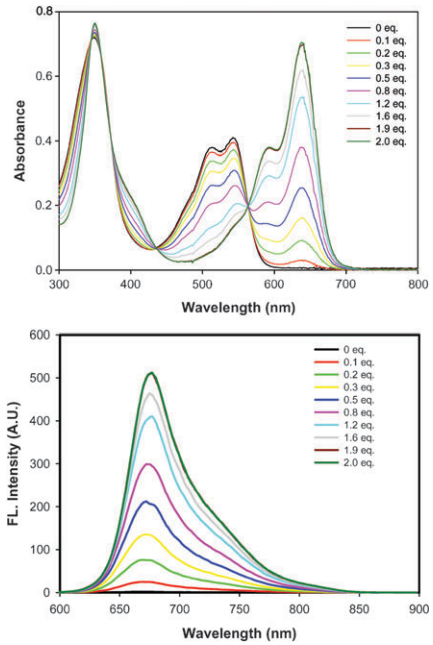


Fig. 1 UV-Vis spectra (top) and fluorescence spectra (bottom) show changes upon titration of **1** (2×10^{-5} M in acetonitrile) with increasing concentrations of Zn^{2+}. Excitation wavelength for fluorescence spectra is 564 nm, the isosbestic point in UV-Vis.

from the ratio of τ_s/τ_r, where τ_s is the fluorescence lifetime obtained from fluorescence decay recorded by means of time-correlated single photon counting and the radiative lifetime, τ_r, is calculated using the Strickler–Berg relation.[32] Using the same method, fluorescence quantum yields of 0.21 and 0.05 were obtained for **Cd·1** and **Hg·1**, respectively. In comparison, the fluorescence quantum yields for 5,10,15,20-tetraphenylporphyrin free base and its zinc complex were reported to be 0.11 and 0.033, respectively.[33] Importantly, chelation turn-on resulted in fluorescence enhancement to a level that was higher than what was previously observed for other zinc specific chemosensors.[10,27,29,34,35] Based on Job plot analysis, compound **1** forms a 1 : 1 complex with Zn^{2+} (see ESI†)

A spectrophotometric titration of **1** with Zn^{2+} is included in Fig. 1. The sharp change in absorption spectra for **1** after the addition of Zn^{2+} demonstrates the perturbation of the electronic structure of compound **1**. Two isosbestic points are observed at λ_{max} = 438 and 564 nm, suggesting a neat complexation process. Incremental addition of Zn^{2+} also resulted in fluorescence enhancement that saturated at the 1.9 equiv. range when 20 μM of **1** were used for titration (Fig. 1 bottom). The increment of UV-Vis spectrum (Fig. 1 top) was used to obtain the stability constant in a Hill plot, as shown in Fig. 2. This analysis provided a Hill plot coefficient of 1 and a stability constant of 2.05×10^5. The large stability constant suggests that **1** can be used to detect Zn^{2+} at low concentrations.

The selectivity of compound **1** for Zn^{2+} makes it a reliable reagent for future applications in biological systems. Fig. 3 shows the fluorescence intensities of **1** treated with physiologically important metal ions. The black bars in the upper panel and the photograph inserted in the lower panel of Fig. 3

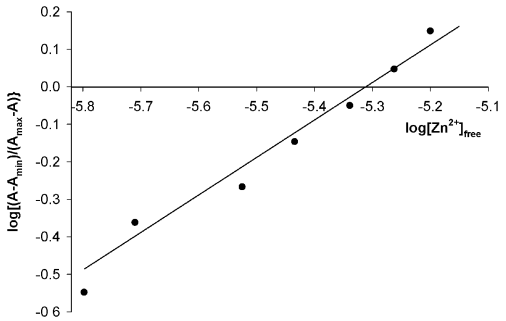


Fig. 2 Hill plot from the spectrophotometric titration in Fig. 1 (top). The data fitting to a straight line with a slope of 1 gives an intercept of 5.312.

demonstrate that only Zn^{2+}, Hg^{2+} and Cd^{2+} turn on fluorescence, whereas other physiologically important cations such as Na^+, Mg^{2+} and Ca^{2+} do not. As is observed for most of Zn^{2+} chemosensors,[29] Cd^{2+} and Hg^{2+} bind to **1** and enhance fluorescence, but to a lesser degree than zinc. The red-shift of $\lambda_{emission}^{max}$ from 672 nm for Zn^{2+} to 682 and 706 nm for Cd^{2+} and Hg^{2+}, respectively, in acetonitrile solution suggests that colorimetric assay can differentiate the three fluorescence turn-on metal ions. The ratio of fluorescence intensity for Zn^{2+}, Cd^{2+} and Hg^{2+} is 17 : 6 : 1, which is large compared to other Zn^{2+} chemosensors.[36,37] Since the concentrations of Cd^{2+} and Hg^{2+} in healthy cells are low, these ions should not interfere with the probing of Zn^{2+}.

Two competitive analyses were carried out to further understand the selectivity of **1** for Zn^{2+} using either **1** in the presence of a variety of metal ions, treated with Zn^{2+}, or using a solution of **Zn·1** complex treated with other metal ions. As indicated by red and green bars shown in Fig. 3, a significant level of fluorescence enhancement was observed for zinc ion

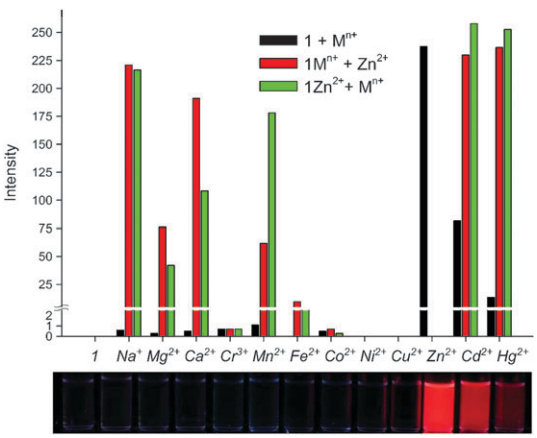


Fig. 3 Top: Fluorescence intensities after treating **1** in acetonitrile with a variety of metal chlorides, and relative binding abilities of **1** to different metal ions compared to Zn^{2+} (excitation at 600 nm). *Conditions*: Black bar, 3×10^{-6} M of **1** with 20 equiv. of metal ion. Red bar, 3×10^{-6} M of **1** in 20 equiv. of metal ion with 20 equiv. of Zn^{2+} then added. Green bar, 3×10^{-6} M of **1** in 20 equiv. of Zn^{2+} with 20 equiv. of metal ion then added. Metal ions were dissolved in water–methanol (1 : 1) at 298 K. Bottom: The fluorescence image of a solution of **1** plus mixed metal chlorides excited by a commercially available UV lamp (λ = 365 nm).

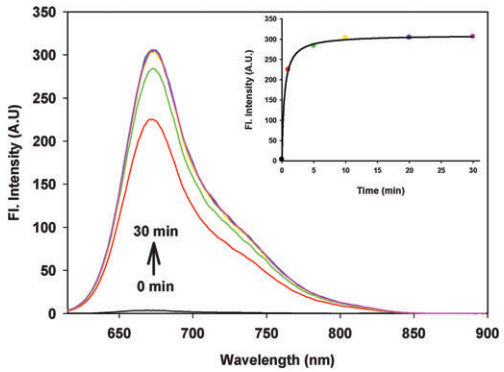


Fig. 4 The changes in fluorescence intensity when 2×10^{-4} M of **1** in CH_3CN in the presence of 3×10^{-3} M 2,6-lutidine was added a mixed solution of metal chlorides (NaCl, $MgCl_2$, $CaCl_2$, $CrCl_3$, $MnCl_2$, $FeCl_2$, $CoCl_2$, $NiCl_2$, $CuCl_2$ and $ZnCl_2$) in methanol to give a stock solution with a concentration of 1×10^{-5} M for each metal ion. The stock solution was diluted 10 times before measurement to give a suitable absorbance in 600 nm, the excitation wavelength.

when competing with most metal ions. The highly elevated fluorescence intensity in the presence of Zn^{2+} where binding was competitive for Cd^{2+} and Hg^{2+} shows that Zn^{2+} forms a more stable complex and can readily replace these ions in the **Cd·1** or **Hg·1** complexes. Among the tested metal ions, Cu^{2+}, Cr^{3+} and Ni^{2+}, which bind strongly to **1** and quench fluorescence because of their paramagnetic effect, interfere with Zn^{2+} detection. However, these ions did not provide a false positive signal that would mimic the presence of Zn^{2+}, and they are not typically present at high concentrations in biological systems.[38] To further evaluate the interference of metal ions to zinc sensing, the solution of **1** was added a solution containing all the metal ions tested in Fig. 3, except Cd^{2+} and Hg^{2+}. The results in Fig. 4 demonstrated a rapid fluorescence response with the intensity reaching a plateau after 5 min.

In conclusion, we show that *m*-benziporphodimethene, a first-generation porphyrin analogue Zn^{2+} chemosensor, is non-fluorescent in the free-base form and exhibits fluorescence turn-on when bound to Zn^{2+}. This sensor exhibits an unusual low energy absorption maximum at 594 nm and an emission wavelength at 672 nm. It is interesting to see that **1**, a simple porphyrin analogue, can readily be used as a zinc-specific sensor with no background fluorescence, and with long wavelengths in both excitation and emission λ_{max}. Its utility in photophysical applications and physiological imaging are foci of ongoing work.

We thank the National Science Council of Taiwan for research funding, researchers at the Mass Spectrometry Center of the Institute of Chemistry at Academia Sinica for the mass spectra measurements and the National Center for High-Performance Computing for use of the computation facilities.

Notes and references

‡ **Zn·1**: ^{1}H NMR (400 MHz, acetone-d_6, 298 K): δ 1.97 (s, 6H; *meso*-CH_3), 2.14 (s, 6H; *meso*-CH_3), 6.05 (s, 2H; 13,14-H), 6.77 (d, ^3J(H,H) = 4.70, 2H; 9,18-H), 6.99 (d, ^3J(H,H) = 4.70, 2H; 8,19-H), 7.31–7.53 (m, 13H; 2,3,4-H, *meso*-phenyl), 8.69 (s, 1H; 22-H); UV-Vis (CH_3CN) [λ_{max}/nm (log ε)]: 350 (4.58), 593 (4.29), 639 (4.56); Anal. (%). Found (calc for $C_{38}H_{32}N_3ZnCl$): C 71.38 (72.48); H 5.57 (5.13); N 5.85 (6.68); HR FAB-MS (*m*/*z*): calc. $[M - Cl]^+$ = 594.1888; obs. $[M - Cl]^+$ = 594.1880.

§ CCDC 662990. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b714412a

1 R. Meallet-Renault, R. Pansu, S. Amigoni-Gerbier and C. Larpent, *Chem. Commun.*, 2004, 2344–2345.
2 L. Fabbrizzi, M. Licchelli, P. Pallavicini, A. Perotti, A. Taglietti and D. Sacchi, *Chem.–Eur. J.*, 1996, **2**, 75–82.
3 C. J. Chang, J. Jaworski, E. M. Nolan, M. Sheng and S. J. Lippard, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 1129–1134.
4 F. Pina, M. A. Bernardo and E. Garcia-Espana, *Eur. J. Inorg. Chem.*, 2000, 2143–2157.
5 P. Jiang and Z. Guo, *Coord. Chem. Rev.*, 2004, **248**, 205–229.
6 R. Kramer, *Angew. Chem., Int. Ed.*, 1998, **37**, 772–773.
7 A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher and T. E. Rice, *Chem. Rev.*, 1997, **97**, 1515–1566.
8 L. Fabbrizzi, M. Licchelli, P. Pallavicini, L. Parodi and A. Taglietti, *Perspect. Supramol. Chem.*, 1999, **5**, 93–134.
9 C. C. Woodroofe, R. Masalha, K. R. Barnes, C. J. Frederickson and S. J. Lippard, *Chem. Biol.*, 2004, **11**, 1659–1666.
10 M. Taki, J. L. Wolford and T. V. O'Halloran, *J. Am. Chem. Soc.*, 2004, **126**, 712–713.
11 P. Chavez-Crooker, N. Garrido and G. A. Ahearn, *J. Exp. Biol.*, 2001, **204**, 1433–1444.
12 W. Breuer, S. Epsztejn, P. Millgram and I. Z. Cabantchik, *Am. J. Physiol.*, 1995, **268**, C1354–C1361.
13 R. Corradini, A. Dossena, G. Galaverna, R. Marchelli, A. Panagia and G. Sartor, *J. Org. Chem.*, 1997, **62**, 6283–6289.
14 Y. Zheng, K. M. Gattas-Asfura, V. Konka and R. M. Leblanc, *Chem. Commun.*, 2002, 2350–2351.
15 A. Torrado, G. K. Walkup and B. Imperiali, *J. Am. Chem. Soc.*, 1998, **120**, 609–610.
16 W. Y. Lin and H. E. Van Wart, *J. Inorg. Biochem.*, 1988, **32**, 21–38.
17 V. Dujols, F. Ford and A. W. Czarnik, *J. Am. Chem. Soc.*, 1997, **119**, 7386–7387.
18 T. Gunnlaugsson, J. P. Leonard, K. Senechal and A. J. Harte, *Chem. Commun.*, 2004, 782–783.
19 G. Klein, D. Kaufmann, S. Schurch and J.-L. Reymond, *Chem. Commun.*, 2001, 561–562.
20 J. J. R. Frausto da Silva and R. J. P. Williams, *The Biological Chemistry of the Elements*, Oxford University Press, New York, 2nd edn, 2001.
21 S. Y. Assaf and S. H. Chung, *Nature*, 1984, **308**, 734–736.
22 J. M. Berg and Y. Shi, *Science*, 1996, **271**, 1081–1085.
23 N. C. Lim, L. Yao, H. C. Freake and C. Bruckner, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 2251–2254.
24 E. M. Nolan, J. W. Ryu, J. Jaworski, R. P. Feazell, M. Sheng and S. J. Lippard, *J. Am. Chem. Soc.*, 2006, **128**, 15517–15528.
25 R. A. Bozym, R. B. Thompson, A. K. Stoddard and C. A. Fierke, *ACS, Chem. Biol.*, 2006, **1**, 103–111.
26 R. Yang, K. a. Li, K. Wang, F. Zhao, N. Li and F. Liu, *Anal. Chem.*, 2003, **75**, 612–621.
27 T. Hirano, K. Kikichi, Y. Urano, T. Higuchi and T. Nagano, *Angew. Chem., Int. Ed.*, 2000, **39**, 1052–1054.
28 K. R. Gee, Z. L. Zhou, W. J. Qian and R. Kennedy, *J. Am. Chem. Soc.*, 2002, **124**, 776–778.
29 S. C. Burdette, C. J. Frederickson, W. Bu and S. J. Lippard, *J. Am. Chem. Soc.*, 2003, **125**, 1778–1787.
30 M. Stepien, L. Latos-Grazynski, L. Szterenberg, J. Panek and Z. Latajka, *J. Am. Chem. Soc.*, 2004, **126**, 4566–4580.
31 D. W. Thomas and A. E. Martell, *J. Am. Chem. Soc.*, 1956, **78**, 1338–1343.
32 S. J. Strickler and R. A. Berg, *J. Chem. Phys.*, 1962, **37**, 814–822.
33 P. G. Seybold and M. Gouterman, *J. Mol. Spectrosc.*, 1969, **31**, 1–13.
34 C. J. Fahrni and T. V. O'Halloran, *J. Am. Chem. Soc.*, 1999, **121**, 11448–11458.
35 S. C. Burdette and S. J. Lippard, *Inorg. Chem.*, 2002, **41**, 6816–6823.
36 Y. Wu, X. Peng, B. Guo, J. Fan, Z. Zhang, J. Wang, A. Cui and Y. Gao, *Org. Biomol. Chem.*, 2005, **3**, 1387–1392.
37 X.-A. Zhang, K. S. Lovejoy, A. Jasanoff and S. J. Lippard, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 10780–10785.
38 N. C. Lim and C. Brueckner, *Chem. Commun.*, 2004, **12**, 1094–1095.

